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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
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SUBJECT: Dividend (Difenoconazole) Qualitative Risk Assessment Based On Charles River CD-1 Mouse Dietary Study

Caswell No. 955

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Summary

This qualitative risk assessment of Dividend (Difenoconazole) was based upon a chronic carcinogenicity feeding study conducted in Charles River CD-1 mice. The study design specified doses of 0, 10, 30, 300, 3000, or 4500 ppm of Dividend for 78 weeks. The males received actual doses of 0, 1.51, 4.65, 46.29, 423.16, or 818.87 mg/kg/day. All 4500 ppm females died or were sacrificed *in extremis* within the first 2 weeks of the study. The remaining female dose groups received actual doses of 0, 1.90, 5.63, 57.79, or 512.61 mg/kg/day.

The statistical evaluation of mortality indicated a significant increasing trend in mortality with increasing doses of Dividend in male mice. Female mice showed no significant incremental changes in mortality with increasing doses of Dividend.

Male mice had significant dose-related increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas. There was a significant difference in the pair-wise comparison of the 46.29 mg/kg/day dose group with the controls for

hepatocellular adenomas. There were significant differences in the pair-wise comparisons of the 423.16 mg/kg/day dose group with the controls for hepatocellular adenomas and combined adenomas and/or carcinomas. There were significant differences in the pair-wise comparisons of the 818.87 mg/kg/day dose group with the controls for hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas.

Female mice had significant dose-related increasing trends, and significant differences in the pair-wise comparisons of the 512.61 mg/kg/day dose group with the controls, for hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas.

Background

A chronic carcinogenicity feeding study in Charles River CD-1 mice was conducted by Hazleton Laboratories America, Incorporated, Vienna, Virginia, for the Agricultural Division of Ciba-Geigy Corporation, Greensboro, North Carolina, and issued April 3, 1989 (Study No. 483-250; MRID No. 420900-15).

The study design allocated groups of 50 mice per sex to dose levels of 0, 10, 30, 300, 3000, and 4500 ppm of Dividend for 78 weeks. An additional 10 mice per sex per dose were designated for interim sacrifice at week 53. Ten more mice per sex in the control, 3000 and 4500 ppm dose groups were designated as post-recovery animals to be dosed until week 53, then sacrificed at week 57. All 70 of the 4500 ppm females, and 15 of the 70 total 3000 ppm females, died or were sacrificed *in extremis* within the first 2 weeks of the study. The 10 control group animals originally designated as post-recovery animals for sacrifice at week 57 were removed from the control group at week 2 and placed in the 3000 ppm dose group. These 10 replaced 10 of the 15 that died or were sacrificed in extremis within the first 2 weeks of the study in the 3000 ppm dose group and were dosed an additional 2 weeks at the end of the study to allow dosing of all animals on the study a total of 78 weeks. At week 3, the 3000 ppm dose was reduced to 2500 ppm for both males and females for the remainder of the study. The males received actual doses of 0, 1.51, 4.65, 46.29, 423.16, or 818.87 mg/kg/day. The remaining female dose groups received actual doses of 0, 1.90, 5.63, 57.79, or 512.61 mg/kg/day.

Survival Analysis

The statistical evaluation of mortality indicated a significant increasing trend in mortality with increasing doses of Dividend in male mice. Female mice showed no significant incremental changes in mortality with increasing doses of Dividend. See Tables 1 and 2 for mortality test results.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analysis

Male mice had significant increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas, all at $p < 0.01$. There was a significant difference in the pair-wise comparison of the 46.29 mg/kg/day dose group with the controls for hepatocellular adenomas at $p < 0.05$. There were significant differences in the pair-wise comparisons of the 423.16 mg/kg/day dose group with the controls for hepatocellular adenomas and combined adenomas and/or carcinomas, both at $p < 0.05$. There were significant differences in the pair-wise comparisons of the 818.87 mg/kg/day dose group with the controls for hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas, all at $p < 0.01$.

These statistical analyses were based upon Peto's prevalence test since there was a statistically significant positive trend for mortality in male mice with increasing doses of Dividend. See Table 3 for male mouse tumor analysis results.

Female mice had significant dose-related increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 512.61 mg/kg/day dose group with the controls for hepatocellular adenomas ($p < 0.01$), carcinomas ($p < 0.05$), and combined adenomas and/or carcinomas ($p < 0.01$).

These statistical analyses were based upon the Exact trend test since there were small numbers of tumors observed in selected instances. The Fisher's Exact test was used for pair-wise comparisons. See Table 4 for female mouse tumor analysis results.

Table 1. Dividend - Charles River CD-1 Mouse Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks					
Dose (mg/kg/day)	1-52	53[i]	53-56	57[i]	57-80[f]	Total
0	2/70	10/68	1/58	9/57	17/48	20/51 (39)**
1.51	2/60	10/58	0/48	0/48	16/48	18/50 (36)
4.65	2/60	10/58	3/48	0/45	18/45	23/50 (46)
46.29	4/60	10/56	0/46	0/46	22/46	26/50 (52)
423.16	0/70	10/70	0/60	10/60	16/50	16/50 (32)
818.87	13[#]/69[a]	10/56	0/46	10/46	20/36	33/49 (67)**

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[#]All 13 of these animals died within the first 25 weeks of the study; 11 of the 13 died before week 4.

[a]One accidental death at week 39, dose 818.87 mg/kg/day.

[i]Interim sacrifices at weeks 53 and 57.

[f]Final sacrifice at week 79.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Dividend - Charles River CD-1 Mouse Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks					
Dose (mg/kg/day)	1-52	53[i]	53-56	57[i]	57-81[f]	Total
0	2/59[a]	10/57	0/47	0/47	23/47	25/49 (51)
1.90	2/59[b]	10/57	1/47	0/46	11/46	14/49 (29)*
5.63	3/59[c]	10/56	2/46	0/44	15/44	20/49 (41)
57.79	4/60	10/56	1/46	0/45	10/45	15/50 (30)
512.61	10[#]/69[d]	10/59	0/49	10/49	10/39	20/49 (41)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[#]All 10 of these animals died within the first 16 weeks of the study; 9 of the 10 died before week 4.

[a]One accidental death at week 6, dose 0 mg/kg/day.

[b]One accidental death at week 51, dose 1.90 mg/kg/day.

[c]One accidental death at week 19, dose 5.63 mg/kg/day.

[d]One accidental death at week 7, dose 512.61 mg/kg/day.

[i]Interim sacrifices at weeks 53 and 57.

[f]Final sacrifice at week 79 for all animals except the 10 animals in the 512.61 mg/kg/day dose group that were originally in the control group which were sacrificed at week 81.

( )Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. Dividend - Charles River CD-1 Mouse Study

Male Hepatocellular Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)					
	0	1.51	4.65	46.29	423.16	818.87
Adenomas (%)	4/68 (6)	10/57 (18)	8/58 (14)	9/56 (16)	13[a]/70 (19)	20/56 (36)
p =	0.000**	0.053	0.078	0.035*	0.036*	0.000**
Carcinomas (%)	1/68 (1)	0/57 (0)	1/58 (2)	0/56 (0)	5/70 (7)	13[b]/56 (23)
p =	0.000**	-	0.546	-	0.093	0.000**
Combined (%)	5/68 (7)	10/57 (18)	9/58 (16)	9/56 (16)	16/70 (23)	28/56 (50)
p =	0.000**	0.114	0.128	0.061	0.023*	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First adenoma observed at week 53, dose 423.16 mg/kg/day.

[b]First carcinoma observed at week 53, dose 818.87 mg/kg/day.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Dividend - Charles River CD-1 Mouse Study

Female Hepatocellular Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)				
	0	1.90	5.63	57.79	512.61
Adenomas (%)	0/57 (0)	0/56 (0)	0/56 (0)	1/56 (2)	16[a]/59 (27)
p =	0.000**	1.000	1.000	0.496	0.000**
Carcinomas (%)	0/47 (0)	0/45 (0)	1/44 (2)	0/45 (0)	4[b]/39 (10)
p =	0.002**	1.000	0.484	1.000	0.039*
Combined (%)	0/57 (0)	0/56 (0)	1/56 (2)	1/56 (2)	17/59 (29)
p =	0.000**	1.000	0.496	0.496	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53 for adenomas and combined, and before week 58 for carcinomas.

[a]First adenoma observed at week 53, dose 512.61 mg/kg/day.

[b]First carcinoma observed at week 72, dose 512.61 mg/kg/day.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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